Synthesis of Enhanced Lipid Solubility of Rutin Derivatives, Formulation and Development of a Validated Analytical Method

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Abstract

Background

Rutin is available in the market as a topical formulation for the treatment of several diseases such as internal bleeding, hemorrhoids, and varicose veins. However, these gels have low solubility and limited bioavailability due to their decreased lipid solubility. In this study, we aimed to synthesize a potentially novel lipophilic rutin prodrug. The suggested library of these prodrugs of rutin is to change the solubility profile to facilitate rutin transport across the biological barrier and therefore improve drug delivery through topical application.

Method:

Six derivatives of the Rutin based on ester pro-drug strategy were synthesized. The synthesized compounds were formulated into a topical ointment and their permeability through Franz diffusion was measured. UV analytical method was developed in our labs to quantify rutin derivatives both as raw material and in the final dosage form. The analytical method was then validated.

Results

The synthesized derivative esters of the Rutin were successfully achieved. The results of Franz diffusion showed that the transdermal permeability was best for decaacetylated rutin compared to the other esterified rutins. A simple analytical method for the analysis of the formulated rutin ester was developed and validated. Moreover, the formulated ointment of decaacetylated rutin in our research laboratory was found to be stable under stability accelerated conditions.

Conclusion

The Synthesis of potentially more lipophilic give novel rutin prodrugs suitable is for topical formulation. This project provides a synthetic approach for many similar natural products. The research idea and strategy followed in this research project is can be adapted by pharmaceutical and herbal establishments.

1. Introduction

Compounds derived from natural products from plants are of interest to many scientific researchers and pharmaceutical companies [1]. Rutin is a bioactive plant flavonoid that owns significant biological therapeutic uses as its inhibition of free-radical, mediated cytotoxicity, and lipid peroxidation. Furthermore, rutin increases the resistance as well as the permeability of capillary blood vessels [2]. Flavonoids represent a promising group to treat many aliments [3–5]. Interest in these substances has

been encouraged by the potential benefits for human health is due to the antioxidant effects of the polyphenolic compounds present in the plants [6–8].

Rutin chemically is (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) as shown in **Fig. 1**; is a flavonoid glycoside that is widespread in the plant phytochemical compounds [9]. This compound is founded usually in various food sources especially in oranges, grapes, lemons, limes, peaches, berries, and buckwheat [10–12]. Rutin is a fine microcrystalline, yellow powder, hardly soluble in water, alcohol and practically insoluble in chloroform and ether [13]. The medicinal applications of rutin are relatively limited because of its low water solubility (0.125 g/L) which seemed to restrict rutin usage [14].

Rutin has many medicinal benefits such as hypertension treatment. It also builds a protective barrier against infection exerting an antibacterial effect, anti-inflammatory as a result of diminishing the formation of pro-inflammatory mediators, as well as diuretic action was observed for rutin, lowers the intensity of cholesterol in the bloodstream. Rutin can be considered as a remedy for allergies, preventing cataracts and macular degeneration is also reported for rutin compound, disorientation, and senility caused by advancing age. Rutin also helps in sustaining collagen synthesis in the tissue just below the skin to improve the epidermis appearance [15, 16]. Rutin treatment was reported to reduce blood glucose levels when elevated as shown previously in a study demonstrated besides that the onset of cardiovascular complications may be reduced and delayed by controlling some metabolic abnormalities [17–19].

Rutins are poorly absorbed from the gastrointestinal tract and also in an irregular absorption manner, almost because of their very low solubility in water and slow dissolution rate so this point increased scientific attention in improving their solubility and dissolution profiles using the prodrug strategy [20–22].

Developing transdermal prodrug has an increased interest during the last decade because of several advantages. Improvement of an effective means of transdermal delivery will usually improve drug concentrations, decrease systemic distribution, and thereby preventing certain limitations of orally administered products [23]. A new derivative of rutin was also synthesized and investigated for topical application to evaluate the potential usage of this derivative in the dermatology field and to investigate its antioxidant, antimicrobial, anti-inflammatory effects [24].

Prodrug plays a significant role in the discovery and designing of new drugs. Several therapeutic areas wherein this approach can be applied and utilized. Achieving pro-drug preparation will be going to be an emerging approach in years to come. Nowadays huge numbers of drugs are being developed into prodrugs to reduce unwanted characters obviously [25–27].

In recent research done by our team, rutin prodrug was synthesized to improve the rutin solubility by selectively acetylating some of the hydroxyl group. The results were successful and the synthesized rutin showed an improved solubility of at least two-fold [28].

Some other researchers reported the production of many rutin prodrugs such as rutin stearate, rutin butyrate [29], and rutin derivatives containing a 1, 4-pentadien-3-one moiety were also done [30–32].

The main parameters that promote the effects of prodrugs are pharmacokinetics, biopharmaceutics, physicochemical as well as its toxicity and bioactivity. As skin well known as a highly active metabolic organ and includes a multitude of various enzymes that can metabolize a wide range of synthetic and xenobiotic compounds that occurred in nature. Skin can change prodrug to the active parent drug back just when they are in the skin layers. Hydrophilic drugs have poor diffusion through the skin; the structural modification to formulate derivatives with higher lipophilicity usually increases the skin permeation capacity [33]. The penetration of topical drugs is a challenge. Isolated animal skin was used to estimate percutaneous absorption of the prepared prodrug molecules, this step is important to understand the pathways, and driving forces of various factors across the skin barrier, after a formulation was completed and a quality control test was finished. The study of drug permeation by Franz cells is a reproducible method. It is easy to implement in the laboratory. It is an authenticated method to evaluate an *in vitro* drug permeation and has many advantages including few handling of tissues, no continuous sample collecting, and a low amount of drug is needed for analysis [34, 35].

Quantification of the synthesized prodrugs in the finished formulated dosage form needs to be performed by a validated analytical method. Validation of analytical method requires testing of parameters recommended by United States Pharmacopeia (USP), Food and Drug Administration (FDA), and International Conference on Harmonization (ICH). These parameters include Specificity, Precision both Repeatability and Ruggedness, Accuracy, Limit of Detection (LOD), and limit of quantification (LOQ) [36–40].

Herein, we aim to synthesize novel lipophilic rutin prodrugs to obtain a suitable derivative with more improved drug delivery. The synthesized prodrugs were formulated in a topical dosage form and their transpermeability and hydrolysablity were evaluated. Moreover, an analytical method was developed and validated to quantify rutin derivatives both as raw material and in its final dosage form.

2. Methodology

2.1 Reagents

Rutin trihydrate (USP grade) powder was purchased from (MP Biomedicals, USA). Acetone, ethanol (EtOH), dichloromethane (DCM), and n-hexane (Hex) were purchased from (C.S. Company, Haifa). Triethylamine (Et_3N), and diethyl ether were purchased from (Merck Millipore). Butryl anhydride, *N*,*N* Diisopropylethyl amine (DIPEA), dimethylaminopyridine (DMAP), acetyl chloride 98%, benzoic anhydride, isovaleric anhydride, isobutyric anhydride, propionyl chloride, 1- octanol, benzyl alcohol, vaseline, paraffin oil were purchased from sigma-aldrich, Germany.

2.2 Equipments

Sensitive weighing balance (Adventurer®, OHAUS Corporation), hotplate stirrer (Lab tech^{R,} daihasn lab tech co,ltd, India), Rotary Evaporator (MRC, ROVA-100, laboratory equipment manufacturer) and NMR spectra performed for the products using Bruker Avance 500 spectrometer at Jordan University. Water bath and Sonicator (Elmasonic S 70 H, Elma®, Germany). Absorption analysis was conducted on (Spectrophotometer-7315, Jenway, UK) using 10-mm quartz cuvettes. Centrifuge (UNIVERSAL-320, Hettich, Zentrifugen, Germany). Centrifuge-DCS-16-RVT (Prevac, Canada), used for 4°C, melting point apparatus (GALLENKAMP, UK), and Franz cell diffusion apparatus.

2.3 Chemical synthesis

2.3.1 Synthesis of Decaacetylated rutin (Ru-10-OAc)

Compound **(Ru-10-OAc)** was synthesized as published previously by our research groups [41]

2.3.2 Synthesis of decapropionate rutin (Ru-10-Prop)

The decapropionate rutin was synthesized by dissolving rutin (0.4 g, 0.66 mmol) in 20 mL DCM and then propionyl chloride (1.7 mL, 19.6 mmol) and 4,4-dimethylamino pyridine (2.9 g, 23.7 mmol) were added as catalyst. The reaction was then stirred for 18 hours at room temperature. Then, the reaction was washed with water and brine (100 ml x 3) and the organic layer was extracted. Followed by addition of sodium sulfate as drying agent, filtration and evaporation to obtain the crude product. The product was purified using silica gel column chromatography (eluent: Hexane:Ethyl acetate, 2:1, v/v) giving R-10-O-Propioante (90%) as a white powder (R_f: 0.44, Hex:EtOAC 2:1).

^{1}H NMR (500 MHz, $CDCl_3$) : δ 7.90–7.86 (m, 2H, Ar), 7.27 (s, 1H, Ar), 7.24 (dd, *J* = 3.3 Hz, *J* = 1.2 Hz, 1H, Ar), 7.20 (d, *J* = 1.0 Hz, 1H, Ar), 6.67 (dd, *J* = 2.2 Hz, *J* = 1Hz, 1H, OCHO), 5.35 (d, *J* = 7.9 Hz, 1H, OCH□O), 5.24–5.11 (m, 3H, CHO, 2CHO), 5.05–4.99 (m, 3H, OCHO, 2CHO), 4.89 (t, *J* = 9.6 Hz, 2H, 2CHO), 3.61–3.55 (m, 2H, $CHCH_2O$, $CHCH_3$), 3.51–3.43 (m, 2H, $CHCH_2O$), 2.56–2.49 (m, 8H, $4ArOCOCH_2$), 2.23–2.09 (m, 12H, $6OCOCH_2$), 1.08–0.95 (m, 33H, $OCOCH_2CH_3$, CH_3). ^{13}C NMR (125 MHz, DMSO): δ 173.4, 173.3, 173.1, 172.9, 172.5, 172.2, 171.9, 171.8, 171.7, 156.6, 154.6, 154.5, 150.1, 142.2, 136.4, 128.5, 127.6, 124.7, 124.0, 114.9, 98.6, 97.5, 70.1, 69.1, 69.0, 68.9, 66.2, 27.4, 27.3, 27.2, 27.0, 17.1, 9.5, 9.4, 9.3, 9.1, 9.0.

2.3.3 Synthesis of the isobutyrate rutin (Ru-10-iBu)

The synthesis of the isobutyrate rutin, the rutin (0.4 g, 0.66 mmol) was dissolved in 15 mL of DCM, then DMAP (0.6 g, 4.91 mmol), triethylamine (0.69 mL, 4.91 mmol) and isobutyric anhydride (3.26 mL, 19.7 mmol) were added. The reaction was stirred at room temperature overnight. Then the reaction was washed with DCM (200 mL x 3) and HCl 1M (100 mL x 3). The organic layers were collected and dried using anhydrous Na_2SO_4. The crude product was purified using silica gel column chromatography (eluent: Hexane/Ethyl acetate, 4/1, v/v) giving R-10-O-*i*Buterate (98%) as a brown powder (R_f: 0.48, Hex:EtOAC 4:1).

^{1}H NMR (500 MHz, DMSO): δ 7.99–7.97 (m, 2H, Ar), 7.50 (d, *J* = 2.4 Hz, 1H, Ar), 7.38 (d, *J* = 9.2 Hz, 1H, Ar), 7.08 (d, *J* = 2.1 Hz, 1H, Ar), 5.69 (d, 1H, *J* = 7.9 Hz, OCHO), 5.49 (t, 1H, *J* = 9.6 Hz, OCH□O), 5.01–4.94 (m, 3H, CHO, 2CHO), 4.89–4.84 (m, 3H, OCHO, 2CHO), 4.76 (t, 1H, *J* = 10.1 Hz, CHO), 4.48 (s, 1H, CHO), 3.98–2.94 (m, 2H, $CHCH_2O$, $CHCH_3$), 3.57–3.54 (m, 2H, $CHCH_2O$), 2.89–2.76 (m, 5H, $5CH(CH_3)_2$), 2.40–2.21 (m, 5H, $5CH(CH_3)_2$), 1.06–0.88 (m, 63H, $10CH(CH_3)_2$, CH_3). ^{13}C NMR (125 MHz, DMSO): δ 175.7, 175.6, 175.4, 175.3, 175.2, 174.8, 174.5, 174.2, 174.0, 171.7, 156.6, 154.6, 150.2, 144.6, 142.2, 136.3, 128.6, 127.4, 124.9, 123.9, 115.1, 114.7, 110.1, 98.7, 97.6, 72.2, 72.0, 71.9, 70.0, 69.2, 68.8, 66.3, 33.9, 33.8, 33.7, 33.6, 33.5, 19.4, 19.1, 19.0, 18.9, 18.8, 18.7, 17.2.

2.3.4 Synthesis of the butyrate rutin (Ru-10-Bu)

The synthesis of the butyrate rutin, the rutin (0.4 g, 0.66 mmol) was dissolved in 15 mL of DCM, then DMAP (0.52 g, 4.27 mmol), triethylamine (0.6 mL, 4.27 mmol) and butyric anhydride (3.21 mL, 20.3 mmol) were added. The reaction was stirred at room temperature for 24 hours. Then the reaction was washed with DCM (200 mL x 3) and HCl 1M (100 mL x 3). The organic layers were collected and dried with anhydrous Na_2SO_4. The crude product was purified using silica gel column chromatography (eluent: Hexane/Ethyl acetate, 1/1, v/v) giving R-10-O-Buterate (93%) as a brown powder (R_f: 0.19, Hex:EtOAC 1:1).

^{1}H NMR (500 MHz, DMSO): δ 8.03–7.99 (m, 2H, Ar), 7.55 (d, 1H, *J* = 2.1 Hz, Ar), 7.43 (d, 1H, *J* = 8.5 Hz, Ar), 7.11 (d, 1H, J = 2.1 Hz, Ar), 5.73 (d, 1H, *J* = 7.6 Hz, OCHO), 5.48 (t, 1H, *J* = 9.6 Hz, OCH□O), 5.05–4.99 (m, 3H, CHO, 2CHO), 4.93–4.88 (m, 3H, OCHO, 2CHO), 4.77 (t, 2H, *J* = 9.7 Hz, 2CHO), 3.98–3.94 (m, 2H, $CHCH_2O$, $CHCH_3$), 3.57–3.53 (m, 2H, $CHCH_2O$), 2.69–2.58 (m, 8H, $4ArOCOCH_2$), 2.32–2.05 (m, 12H, $6OCOCH_2$), 1.74–1.41 (m, 20H, $10OCOCH_2CH_2$), 1.04–0.80 (m, 33H, $OCOCH_2CH_2CH_3$, CH_3). ^{13}C NMR (125 MHz, DMSO): δ 174.8, 172.4, 172.2, 172.1, 172.0, 171.8, 171.6, 171.2, 170.9, 170.7, 156.6, 154.5, 150.0, 144.4, 142.1, 136.4, 128.5, 127.5, 124.8, 124.0, 115.0, 114.7, 110.1, 97.5, 72.1, 71.9, 71.8, 70.0, 69.0, 68.9, 66.5, 66.2, 36.0, 35.8, 35.7, 35.6, 35.5, 35.4, 18.5, 18.4, 18.3, 18.2, 18.1, 18.0, 17.2, 14.0, 13.8, 13.7, 13.6.

2.3.5: Synthesis of the isovaleric rutin (Ru-10-O-ⅰ Valerate)

The synthesis of the isovalerate rutin, the rutin (0.4 g, 0.66 mmol) was dissolved in 15 mL of DCM, then DMAP (0.6 g, 4.91 mmol), triethylamine (0.91 mL, 6.45 mmol) and isovaleric anhydride (4.00 mL, 20.01 mmol) were added. The reaction was stirred at room temperature for 18 hours. Then the reaction was washed with DCM (200 mL x 3) and HCl 1M (100 mL x 3). The organic layers were collected and dried with anhydrous Na_2SO_4. The crude product was purified using silica gel column chromatography (eluent: Hexane/Ethyl acetate, 4/1, v/v) giving R-10-O-*N*valerate (91%) as a brownish oil (R_f: 0.55, Hex:EtOAC 4:1).

^{1}H NMR (500 MHz, DMSO): δ 8.02-8.00 (m, 2H, Ar), 7.52 (d, *J* = 2.4 Hz, 1H, Ar), 7.42 (d, *J* = 8.9 Hz, 1H, Ar), 7.10 (d, *J* = 2.1 Hz, 1H, Ar), 5.70 (d, 1H, *J* = 7.9 Hz, OCHO), 5.49 (t, 1H, *J* = 9.5 Hz, OCH□O), 5.06–4.98 (m, 3H, CHO, 2CHO), 4.92–4.88 (m, 3H, OCHO, 2CHO), 4.79 (t, 1H, *J* = 10.1 Hz, CHO), 4.50 (s, 1H, CHO), 3.97–

2.93 (m, 2H, $CHCH_2O$, $CHCH_3$), 3.57–3.52 (m, 2H, $CHCH_2O$), 2.23–1.84 (m, 30H, 10 $CH_2CH(CH_3)_2$,10$CH_2CH(CH_3)_2$), 1.05–0.80 (m, 63H, 10$CH(CH_3)_2$, CH_3). ^{13}C NMR (125 MHz, DMSO): δ 176.2, 175.9, 175.6, 175.4, 175.3, 174.9, 174.7, 174.6, 174.5, 172.2, 157.1, 155.6, 150.5, 144.4, 142.2, 136.3, 128.6, 127.4, 124.9, 123.9, 115.1, 114.7, 110.5, 98.6, 97.9, 72.2, 72.1, 71.7, 70.0, 69.0, 67.8, 66.5, 44.9, 44.8, 44.7, 44.6, 44.5, 33.9, 33.9, 33.7, 33.6, 33.4, 19.5, 19.2, 19.1, 19.0, 18.8, 18.7, 17.2.

2.3.6 Synthesis of the benzoate rutin (Ru-10-O-Benzoate)

The rutin (0.4 g, 0.66 mmol) was dissolved in 15 mL of DCM, then DMAP (0.6 g, 4.91 mmol), triethylamine (0.91 mL, 6.45 mmol) and benzoic anhydride (4.45 g, 19.7 mmol) were added. The reaction was stirred at room temperature for 18 hours. Then the reaction was washed with DCM (200 mL x 3) and HCl 1M (100 mL x 3). The organic layers were collected and dried with anhydrous Na_2SO_4. The crude product was purified using silica gel column chromatography (eluent: Hexane/Ethyl acetate, 4/1, v/v) giving R-10-O-benzoate (90%) as a white powder (R_f: 0.2, Hex:EtOAC 2:1).

^1H NMR (500 MHz, DMSO): δ 8.45–8.42 (m, 2H, Ar), 8.28 (d, *J* = 7.9 Hz, 2H, Ar), 8.20 (d, *J* = 7.9 Hz, 2H, Ar), 8.11 (d, *J* = 8.2 Hz, 2H, Ar), 8.01 (d, *J* = 7.9 Hz, 2H, Ar), 7.96–7.23 (m, 45H, Ar), 6.20–6.13 (m, 2H, 2OCHO), 5.66 (t, 1H, *J* = 8.5 Hz, CHO), 5.59–5.51 (m, 3H, 3CHO), 5.40 (t, 1H, *J* = 9.5 Hz, CHO), 4.97 (s, 1H, CHO), 4.58 (bs, 1H, CHO), 4.12 (m, 1H, $CHCH_2O$), 3.92 (d, 1H, *J* = 10.4 Hz, CHO), 3.72–3.68 (m, 1H, $CHCH_2O$), 1.09 (d, *J* = 6.1 Hz, CH_3). ^{13}C NMR (125 MHz, DMSO): δ 171.8, 167.8, 165.6, 165.5, 165.3, 165.0, 164.9, 164.8, 164.1, 164.0, 163.8, 156.6, 155.0, 154.5, 150.0, 144.7, 142.7, 136.8, 135.0, 134.8, 134.5, 134.3, 134.2, 134.0, 133.3, 131.3, 130.6, 130.5, 130.2, 130.1, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.6, 128.4, 128.3, 127.6, 124.4, 115.5, 110.4, 99.1, 98.1, 73.3, 72.9, 72.8, 71.7, 70.3, 70.2, 66.6, 36.7, 24.8, 24.2, 21.2, 17.6.

2.4 Topical ointment Formulation:

The ointment was prepared by weighing 8.2 g Vaseline as a base, 1.25 g paraffin oil as a base, and was mixed at 75°C. The active ingredient (rutin derivative, 0.050 g) and 0.5 g benzyl alcohol were dissolved and added to the melted Vaseline and paraffin oil mixture. The reaction mixture was then left to cool down to room temperature. Two more ointment formulations were prepared in the laboratory in which the amount of benzyl alcohol (penetration enhancer) was varied (0.25 g and 0.75g) while keeping the other compositions the same. The amount change of benzyl alcohol was adjusted by changing the Vaseline amount to have a net weight of 10 g. The intentional variation in the new two formulations was done to optimize the best diffusion through the skin.

Four different active ingredients were formulated into an ointment. These ingredients were: decaacetylated rutin, decaisobuterate rutin, decapropionate rutin, and decabuterate rutin.

2.5 Diffusion determination of the Rutin and its derivatives through Franz diffusion cell

The diffusion through the skin of the rutin derivative in the ointment formulation of a dissected mouse was examined using Franz diffusion cell.

A fresh skin from either the abdomen or the back of the mouse was installed on Franz diffusion cell apparatus in our research Laboratory. A definite amount of the ointment was weighed and applied to the skin of the mouse after filling the receptor chamber with ethyl alcohol **(**Table 1**).** The donor chamber is placed over the membrane and sealed with a clamp. Samples were then withdrawn every half hour for six hours. The same experiment was repeated three times for each formulation and the average reading was taken for the calculation.

The concentration of the released active ingredient was calculated using the absorbance at maximal absorption (λ_{max}) determined previously for each active ingredient and applying the regression line equation generated for the calibration curve.

Table 1
Weights applied on the skin of the Franz diffusion

Ointment	Weight (gm)
Decaacetylate rutin	0.2149
Decabuterate rutin	0.226
Decaisobuterate rutin	0.135
Decapropionate rutin	0.123
Original rutin	0.235

2.6 Analytical Method development and validation

Analytical method development using UV-Vis spectrophotometer was done for the Decaacetylated rutin ester in an ointment dosage form.

At first, the Decaacetylated rutin ester was dissolved in ethyl alcohol and was diluted to a sufficient quantity with ethanol, the UV absorbance in the range of 200–600 nm was scanned and recorded to determine the wavelength of maximal absorption (λ_{max}).

The selectivity of the wavelength (λ_{max}) absorption to the active ingredient in the ointment was performed by scanning Decaacetylated rutin UV-Vis absorbance spectrum in the range (200–600 nm) in presence of benzyl alcohol. The synthesized rutin prodrug 0.1 mg/ml was dissolved in ethyl alcohol and was placed in a 25 ml volumetric flask. Two drops of benzyl alcohol were added to the solution and the volume was then completed to 25 ml. The mixture was allowed to shake for 1 minute. The solution mixture was then scanned in the range (200–600 nm) to determine the (λ_{max}).

2.6.1 Method of analysis of formulated ointment

Decaacetylated rutin ester ointment (2 g) was weighed and heated until the petroleum jelly and paraffin oil were dissolved. The dissolved ointment was mixed with ethanol (15 mL) in a clean Falcon tube and put it in centrifuge 4000 rpm for 10 minutes to separate it. The sample after being separated was filtered and then the volume was completed to 20 ml. The absorption of the filter solution was measured using UV/Vis device at λmax (320 nm). The actual amount of deccaacetylated rutin was calculated using the established regression line equation of the calibration curve.

2.6.2 Analytical method validation:

Linearity and range

The linearity and range of method were performed by preparing a stock solution (0.2 mg/ml) which was prepared by weighing 20 mg of decaacetylated rutin ester powder and dissolved in 100 ml ethyl alcohol solvent. The stock solution (0.2 mg/ml) was further diluted to prepare five solutions (0.06, 0.08, 0.1, 0.12, 0.14 mg/ml). A calibration curve was constructed using the predetermined maximal absorption (λ_{max}) of the compound. The regression line equation and the R^2 of the diagram were generated using Microsoft excel 2007.

Accuracy and precision

Decaacetylated rutin ester ointment (2 g) was weighed and heated until the petroleum jelly and paraffin oil were dissolved. The mixture was then put in a sterile Falcon tube having ethanol. The mixture was then centrifuged to separate it. The sample mixture after being separated was filtered and the volume was completed to 20 ml with ethanol. The absorption of the filter solution was measured using UV/Vis device at λ_{max} (320 nm). The actual amount of deccaacetylated rutin was calculated using the established regression line equation of the calibration curve.

Robustness of the analytical method

The robustness of the developed analytical method was tested by applying small intentional variations to the method analytical test conditions. These variations involved testing on different days, using different analysts, measuring at different UV/Vis spectrophotometer instrument wavelengths (320 ± 2 nm). The experiment was carried out with a concentration of 0.1 mg/ml.

2.7 Stability indicating study of decaacetylated rutin under different stress conditions:

A stability-indicating study was done using forced degradation which was done by subjecting the active ingredient along with the excipients to stress conditions of acid/base hydrolysis, heat, light, and oxidation. A solution having a concentration of 0.1 mg/mL was subjected to different stress conditions and samples were analyzed frequently; stress testing is stopped if 5–20% degradation is obtained, or when no degradation is observed after the maximum recommended time. Three separate sample

solutions (9 ml) were taken. To the first samples, 1 ml of 1N HCl was added, to the second sample, 1N NaOH, and the third 3% H_2O_2 were added. Other sample solutions were subjected to heat (70 ^{0}C) and UV (254 nm) light. Thus all the samples were examined under stress conditions including thermal, photolytic, oxidizing, acidic, and basic stress conditions.

2.8 *In vitro* hydrolysis test

The synthesized rutin prodrugs (R-10-OAc, R-10-Prop, R-10-Bu, R-10-iBu) were exposed to esterase enzyme to be hydrolyzed. It was achieved by incubating 1 mg of rutin prodrug within 10 mL ethyl alcohol containing 1 mg of esterase enzyme. Samples were taken from the solution for eight hours. Each time an aliquot was taken and replaced with ethyl alcohol to maintain the sink condition. The concentration of the hydrolyzed prodrug was determined using the developed Spectrophotometric method.

3. Result And Discussion

3.1 Chemical synthesis

The synthesis of the fully buterated, benzoated, isobuterated and isovalerated rutin esters was successfully achieved. The synthesis using various anhydrides, DMAP and triethylamine as catalysts. The method is summarized as shown in Scheme 1. The produced rutin esters were obtained in good yields ≥ of 90%. The structures of the products were confirmed by nuclear magnetic resonance.

The synthesis of the fully acetylated and propionate rutin esters was achieved as shown in Scheme 2. The synthesis involved using acyl chlorides and DMAP/ DIPEA to obtain the fully esterified rutins in a good yield ≥ of 84%. The structures of the products were confirmed by nuclear magnetic resonance

3.2 Diffusion of Rutin and a newly synthesis rutin derivative through Franz diffusion cell:

Four rutin ester derivatives were selected for ointment formulation. The selection of the synthesized compounds was based on the lipophilicity and solubility of the synthesized compounds in ethyl alcohol which was used as a solvent in the Franz diffusion experiment. Tow rutin ester derivatives were excluded from ointment formulation. The decaisovalerate and decabenzoate rutin compound were excluded due to its low lipid solubility.

The solubility of the rutin derivatives in ethyl alcohol was good for all the synthesized compounds approximately (10 mg/ml). Moreover, the underivatized rutin solubility in ethanol was (0.8mg/ml); therefore, ethyl alcohol is a suitalbe solvent to be used in Franz diffusion test.

Based on the log P value and the synthetic yield, four derivatives were selected for ointment formulation and their permeability through the skin was tested using Franz diffusion. The Franz diffusion results of the ointment formulated rutin derivatives of decaacetylated, decapropionate, decabuterate, decaisobuterate rutin ointments are shown in Fig. 2.

The results indicate that the diffusion was best for decaacetylated rutin compared to the other esterified rutin compounds. While as expected the rutin transdermal diffusion was almost zero.

3.3 Analytical method development

As the decacetylated rutin ester formulated ointment showed the highest transdermal diffusion; a simple and validated analytical method was developed.

The UV absorbance of decaacetylated rutin in the range of (200–600 nm) showed two wavelengths of maximum absorption at 245 and 320 nm (Fig. 3). It was decided to consider 320 nm as a measuring (λ_{max}) and not 245 nm for specificity and accuracy reasons.

The results showed that there is the selectivity of the specified absorbance wavelength with formulation excipients. The UV absorption scan for Decaacetylated rutin in the presence of benzyl benzoate excipient in the range (200–600 nm) showed no interference with the measuring (λ_{max}).

3.4 Method validation

Linearity and range of decaacetylated rutin ester

The linearity and range of method were done by measuring the absorbance of five prepared test solutions in the range of (0.06–0.14 mg/ml). The concentration (mg/ml) and absorbance of these concentrations are shown in Table 2

Table 2
Ethyl alcohol calibration curve test solution.

Concentration (mg/ml)	Absorbance (nm)
0.06	0.871
0.08	1.058
0.1	1.35
0.12	1.58
0.14	1.803

The calibration curve was constructed using excel 2007 where Y-axis represented the absorbance and X-axis represented the concentration. The calibration curve is shown in Fig. 4. The linearity relationship between UV absorbance and concentration was examined and it showed a linear relationship with R^2 value of 0.996. The regression line equation was: $y = 11.93x + 0.139$.

Accuracy and precision

Decaacetylated rutin ester ointment was analyzed by the developed analytical method using UV/Vis at λ_{max} of 320 nm. The analysis was repeated four times. The concentration of decaacetylated rutin ester in

the ointment was then calculated and compared with the actual concentration; the calculated percentage accuracy and the % RSD are shown in **Table 3**

Table 3: Accuracy and precision:

Concentration (mg/ml)	% Accuracy
0.099627	99.6
0.099031	99
0.099404	99.4
0.099255	99.2
%RSD	**0.25%**

The percentage accuracy and the %RSD results indicate good accuracy and precision of the developed analytical method.

Robustness

Small variations were applied on a number of the analytical method parameters to confirm the robustness of the method. Results shown in Table 4 indicate the stability of the test when the test was done on different days, different analysts, different instruments, and when applying small variations in the measurement wavelength (± 2 nm). The experiment was carried out with a concentration of 00.1 mg /ml.

Table 4
Robustness of the developed UV analytical method

Day to day variations	
Day	**Concentration mg/ml**
Day 1	0.097392
Day 2	0.097541
Day 3	0.097243
RSD%	0.15%
Different analyst	
Analyst 1	0.097392
Analyst 2	0.097168
RSD%	0.16%
Different instrument	
Instrument 1	0.097392
Instrument 2	0.097765
RSD	0.26%
Wavelength variation	
320nm	0.097392
322nm	0.094411
318nm	0.097541
%RSD	1.77%

3.5 Stability indicating study of decaacetylated rutin under different stress conditions:

Decaacetylated rutin solutions were subjected to different stress conditions including 1N NaOH, 1 N HCl, UV light (254 nm), 3% H_2O_2, and 70 ^{0}C temperature. The results under each condition are shown separately in Table 5.

Table 5
Stability of decaacetylated rutin under different stress conditions:

Stress conditions;	Time (hour)	Concentration	Degradation %
Temperature (70^0C)	1	0.063115	63%
	6	0.005365	5.3%
UV (254 nm)	0.5	0.076826	77%
	24	0.014978	15%
1N NaOH	0.5	0.075261	75%
	24	0.049627	50%
1 N HCl	1	0.096647	96%
	25	0.039344	39%
3% H_2O_2	1	0.098137	98%
	26	0.042027	42%

The stability-indicating results showed that the compound was unstable under all the subjected stress conditions. The main reason explaining the instability of the acetylated rutin ester compound under these stress conditions is the fact that the ester bond is generally unstable. Moreover, the results showed decay in the absorbance of all the measured samples under all the above stress conditions which indicate specificity and selectivity of the analytical method.

3.6 *In vitro* esterase hydrolysis

In vitro esterase hydrolysis of the synthesized rutin prodrugs were investigated. Since various prodrugs of the rutin were synthesized by ester bond formation, these prodrugs were incubated with 1 mg of esterase enzyme to study the percentage of hydrolysis of the ester bond upon a time. As can be observed in Fig. 5, all the synthesized prodrugs have achieved almost complete hydrolysis after seven hours with a half-life of 3.5-4 hours. These results confirm that after the systematic absorption of the prodrugs, they will almost completely convert to the active rutin. Moreover, these data prove that the synthesized ester derivatives didn't impair the activity of the esterase enzyme.

4. Conclusion

A fully esterified decaacetylated rutin, decabuterate rutin, decabenzoate rutin, decaisobuteric rutin, decaisovaleric rutin, decapropionate rutin were synthesized. Decaacetylated showed to have an increased lipid solubility when compared with underivized rutin. The diffusion was best for decaacetylated rutin compared to the other esterified rutin compounds and derivatized rutin. The developed UV/Vis analytical method was successfully used in the assay of decaacetylated rutin ointment and the formulated ointment has shown to be unstable when tested. The ointment formula as well as the developed

analytical method can be readily used by pharmaceutical companies in the formulation and routine quality control of the newly developed rutin derivative.

Declarations

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Ethics approval and consent to participate: The dead animal skin used in the experimental part were provided by An-Najah National University animal house. The animal house provided the items after taking the required approval from the university ethical committee. All the animal experiments and methods were carried out in accordance with ARRIVE guidelines and regulations.

Consent for publication: Not applicable

Competing interests: There is no competing interest

Funding: No funding available

Authors' contributions: All the author reviewed and contributed to the manuscript

Availability of data and materials: The datasets acquired and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Lahlou M. Screening of natural products for drug discovery. Expert Opinion on Drug Discovery 2007;2:697–705.
2. Ganeshpurkar A, Saluja AK. The Pharmacological Potential of Rutin. Saudi Pharmaceutical Journal 2017;25:149–64.
3. Brodowska KM. Natural flavonoids: classification, potential role, and application of flavonoid analogues. European Journal of Biological Research 30/6/2017:108 – 23.
4. Vinson JA, Dabbagh YA. Tea phenols: Antioxidant effectiveness of teas, tea components, tea fractions and their binding with lipoproteins. Nutrition Research 1998;18:1067–75.
5. Sunada S, Fujisawa H, Cartwright IM, Maeda J, Brents CA, Mizuno K, Aizawa Y, Kato TA, Uesaka M. Monoglucosyl-rutin as a potential radioprotector in mammalian cells. Molecular Medicine Reports 2014;10:10–4.
6. Duthie G, Crozier A. Plant-derived phenolic antioxidants. Current Opinion in Lipidology 2000;11:43–7.

7. Kumar S, Mishra A, Pandey AK. Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models. BMC Complementary and Alternative Medicine 2013;13.
8. Karak P, Department of Physiology BCC, Bankura – 722101, West Bengal, India. BIOLOGICAL ACTIVITIES OF FLAVONOIDS: AN OVERVIEW. International Journal of Pharmaceutical Sciences and Research 2019;10.
9. Hosseinzadeh H, Nassiri-Asl M. Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. Journal of Endocrinological Investigation 2014;37:783–8.
10. Kreft S, Knapp M, Kreft I. Extraction of Rutin from Buckwheat (FagopyrumesculentumMoench) Seeds and Determination by Capillary Electrophoresis. Journal of Agricultural and Food Chemistry 1999;47:4649–52.
11. Huang W-y, Zhang H-c, Liu W-x, Li C-y. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. Journal of Zhejiang University SCIENCE B 2012;13:94–102.
12. Abuajah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: a review. J Food Sci Technol 2015;52:2522–9.
13. Serban THG. The isolation and identification of rutin from pharmaceutical products. Researchgate December 2016:109–14.
14. Khalifa TI, Muhtadi FJ, Hassan MMA. Rutin. Analytical Profiles of Drug Substances1983. p. 623–81.
15. Luo H, Jiang BH, King SM, Chen YC. Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nutr Cancer 2008;60:800–9.
16. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MN. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. J Control Release 2006;113:189–207.
17. Russo A, Acquaviva R, Campisi A, Sorrenti V, Di Giacomo C, Virgata G, Barcellona ML, Vanella A. Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. Cell Biology and Toxicology 2000;16:91–8.
18. Pietta P-G. Flavonoids as Antioxidants. Journal of Natural Products 2000;63:1035–42.
19. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation 2007;115:3213–23.
20. Das S, Rosazza JPN. Microbial and Enzymatic Transformations of Flavonoids⊥. Journal of Natural Products 2006;69:499–508.
21. Biely P, Cziszarova M, Wong KK, Fernyhough A. Enzymatic acylation of flavonoid glycosides by a carbohydrate esterase of family 16. Biotechnol Lett 2014;36:2249–55.
22. Lauro MR, Torre ML, Maggi L, De Simone F, Conte U, Aquino RP. Fast- and slow-release tablets for oral administration of flavonoids: rutin and quercetin. Drug Dev Ind Pharm 2002;28:371–9.
23. N'Da DD. Prodrug strategies for enhancing the percutaneous absorption of drugs. Molecules 2014;19:20780–807.

24. Baldisserotto A, Vertuani S, Bino A, De Lucia D, Lampronti I, Milani R, Gambari R, Manfredini S. Design, synthesis and biological activity of a novel Rutin analogue with improved lipid soluble properties. Bioorganic & Medicinal Chemistry 2015;23:264–71.
25. Di L, Kerns EH. Prodrugs. Drug-Like Properties2016. p. 471–85.
26. Ita KB. Prodrugs for transdermal drug delivery – trends and challenges. Journal of Drug Targeting 2016;24:671–8.
27. Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, Savolainen J. Prodrugs: design and clinical applications. Nature Reviews Drug Discovery2008. p. 255–70.
28. Abualhasan M, Assali M, Jaradat N, Sarhan T. Synthesis, Formulation and Analytical Method Validation of Rutin Derivative. Letters in Drug Design & Discovery 2019;16:685–95.
29. Viskupicova J, Danihelova M, Ondrejovic M, Liptaj T, Sturdik E. Lipophilic rutin derivatives for antioxidant protection of oil-based foods. Food Chemistry 2010;123:45–50.
30. Han Y, Ding Y, Xie D, Hu D, Li P, Li X, Xue W, Jin L, Song B. Design, synthesis, and antiviral activity of novel rutin derivatives containing 1, 4-pentadien-3-one moiety. European Journal of Medicinal Chemistry 2015;92:732–7.
31. Stuard S, Cesarone MR, Belcaro G, Dugall M, Ledda A, Cacchio M, Ricci A, Ippolito E, Renzo AD, Grossi MG. Five-year treatment of chronic venous insufficiency with O-(β-hydroxyethyl)-rutosides: Safety aspects. International Journal of Angiology 2011;17:143–8.
32. You HJ, Ahn HJ, Ji GE. Transformation of Rutin to Antiproliferative Quercetin-3-glucoside byAspergillus niger. Journal of Agricultural and Food Chemistry 2010;58:10886–92.
33. Sloan KB. Prodrugs: Topical and Ocular Drug Delivery (Drugs and the Pharmaceutical Sciences) 1st Edition. 1 ed.
34. Simon A, Amaro MI, Healy AM, Cabral LM, de Sousa VP. Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with in vivo-in vitro correlation. International Journal of Pharmaceutics 2016;512:234–41.
35. Kulkarni KN, Datta-Gupta A. Estimating Relative Permeability From Production Data: A Streamline Approach. SPE Journal 2013;5:402–11.
36. R. Lloyd Snyder JJKaLJG. Practical HPLC method development,1997. (2nd Ed) 2007.
37. Br. Jay JK, and B. Pierre. Understanding and Implementing Efficient Analytical Methods Development and Validation. 2003.
38. WHO. Guidelines for Stability Testing of Pharmaceutical Products Containing Well,Established Drug Substances in Conventional Dosage Forms. WHO Expert Committee on Specifications for Pharmaceutical Preparations, Technical Report Series 863: World Health Organization, Geneva 1996,. p. 65–79.
39. ICH. Good Manufacturing Practices for Active Pharmaceutical Ingredients. International Conference on Harmonization, IFPMA, Geneva.; 2000.

40. Alsante KM FR, Hatajik TD, Lohr LL, Sharp TR, Snyder KD, Szczesny EJ. Degradation and impurity analysis for pharmaceutical drug candidates Handbook of Modern Pharmaceutical Analysis,. Academic Press, Boston,(Chap. 4), in: S. Ahuja, S. Scypinski (Eds.); 2001. p. pp.85–172.
41. Murad A, Mohyeddin A, Nidal J, Tala S. Synthesis, Formulation and Analytical Method Validation of Rutin Derivative. Letters in Drug Design & Discovery 2019;16:685–95.

Figures

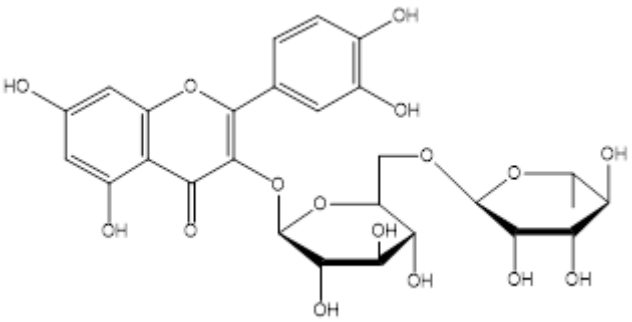

Figure 1

Chemical structure of Rutin.

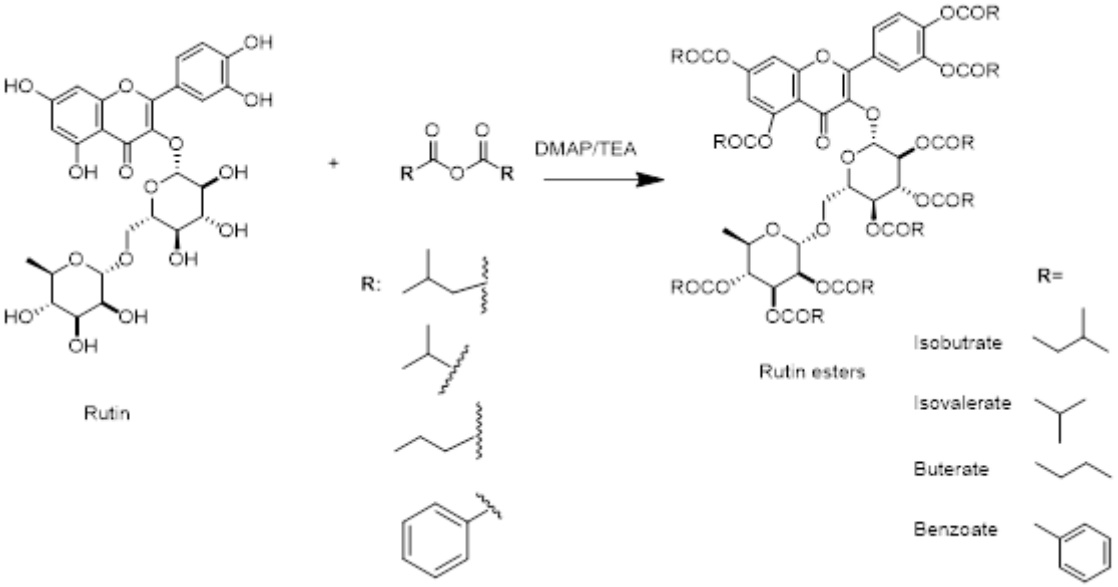

Figure 2

synthesis of buterated, benzoated, isobuterated and isovalerated rutin ester.

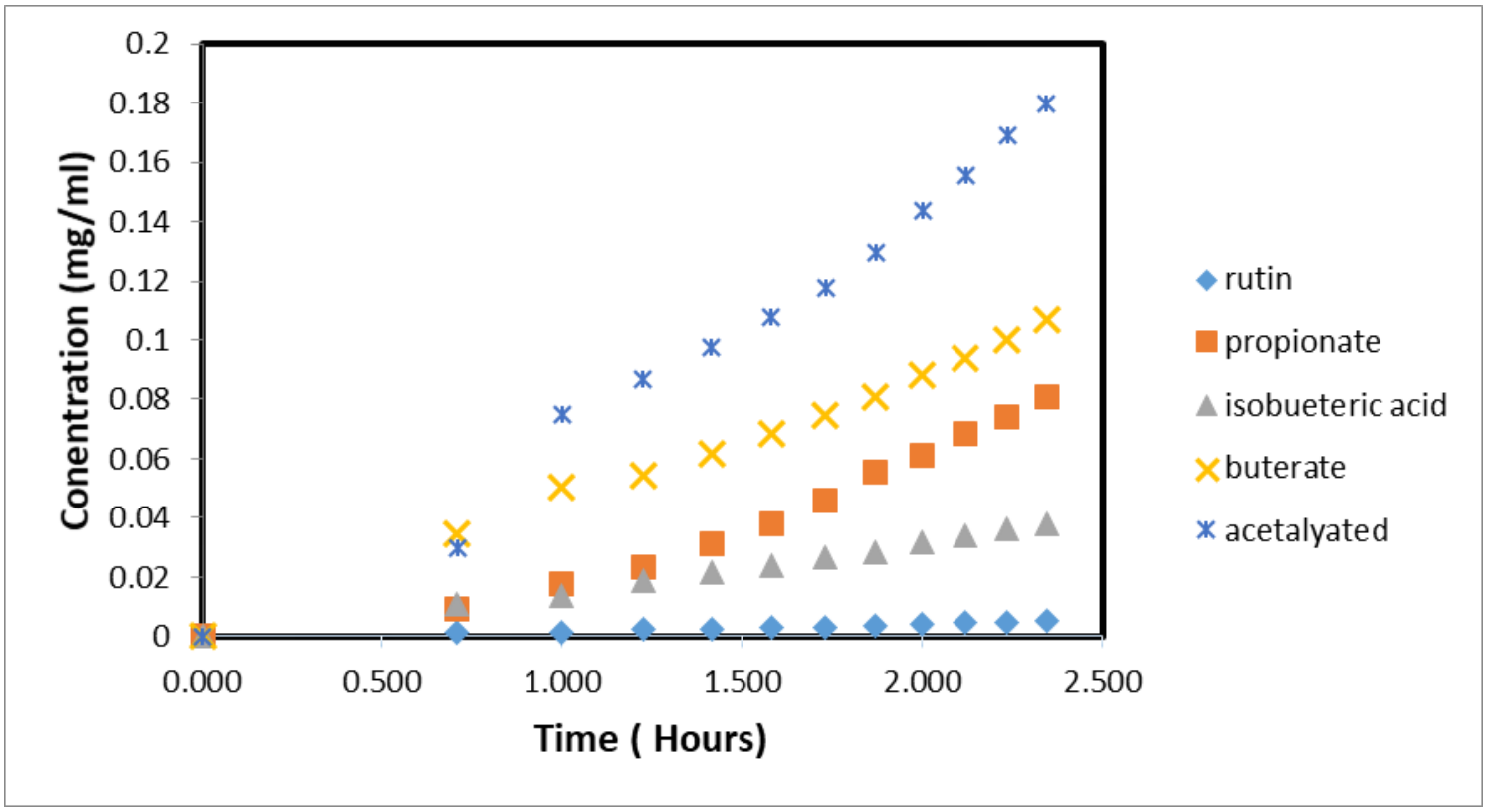

Figure 3

The Franz diffusion results of rutin derivatives in ointment formulation.

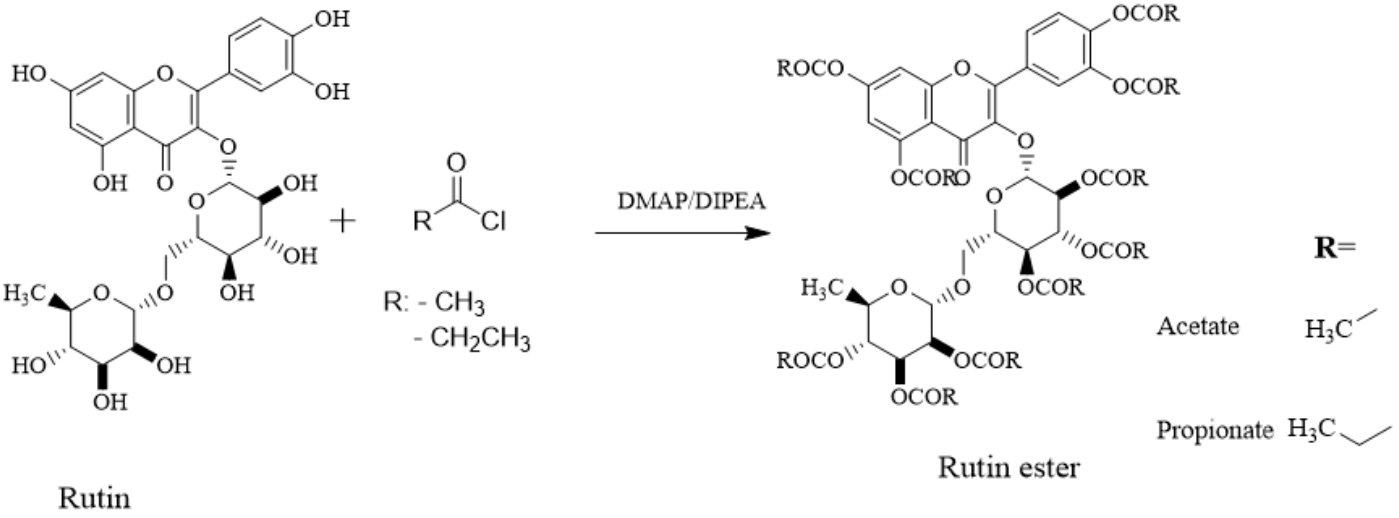

Figure 4

Synthesis of acetylated and propionate rutin esters.

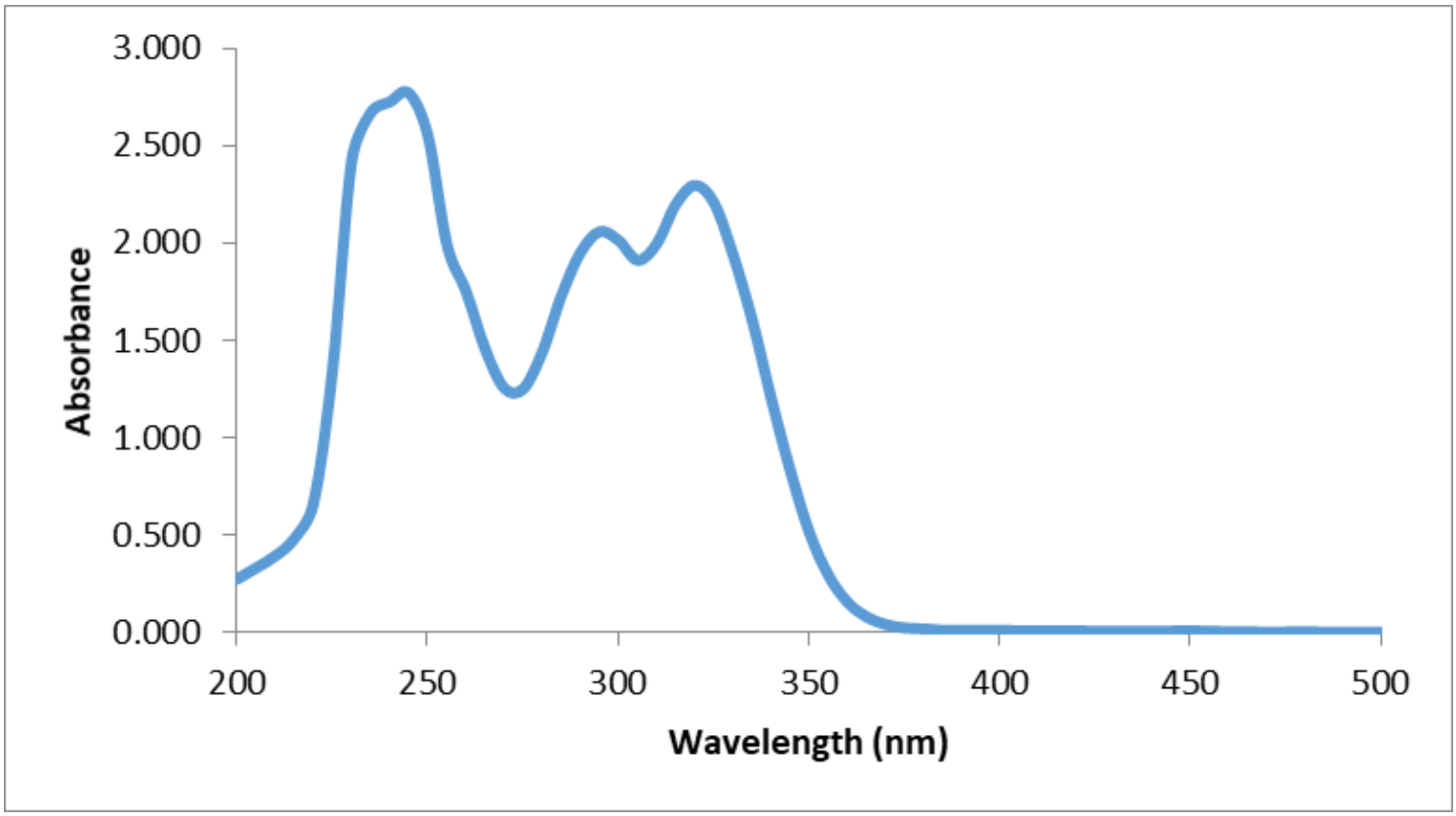

Figure 5

UV-Vis spectrum of decaacetylated rutin sample.

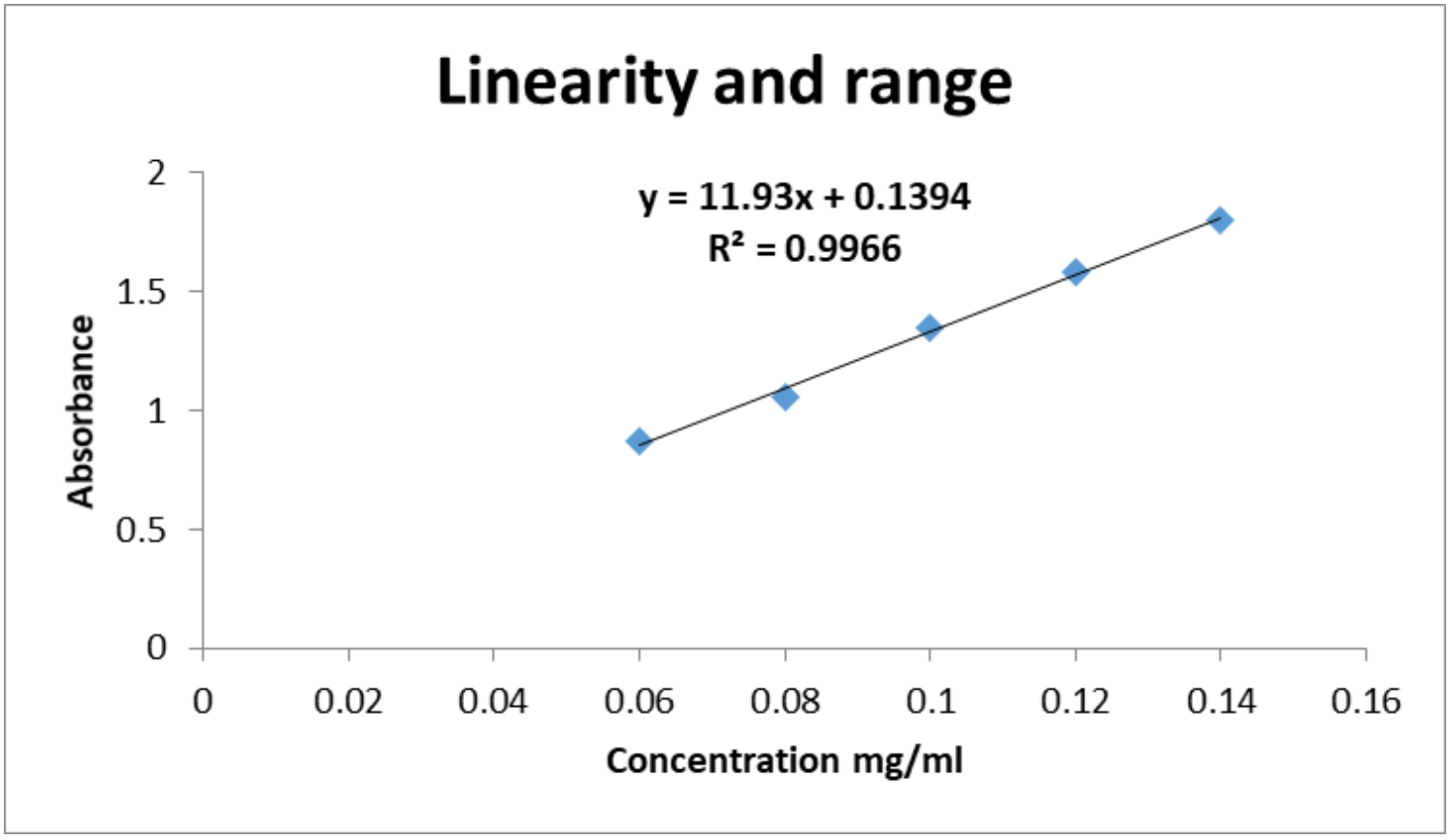

Figure 6

linearity curve of decaacetylated rutin ester.

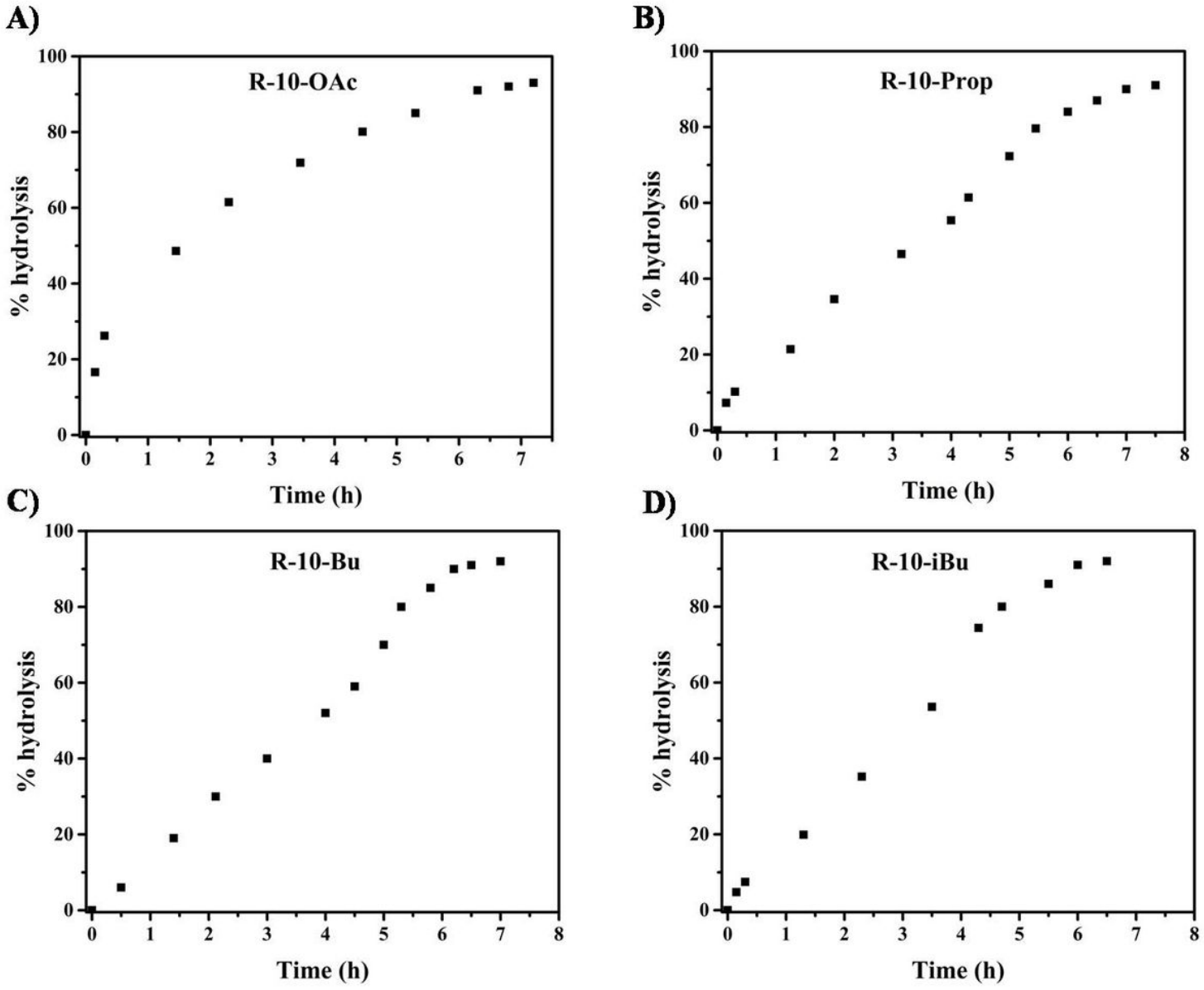

Figure 7

In vitro esterase hydrolysis of A) R-10-OAc; B) R-10-Prop; C) R-10-Bu; D) R-10-iBu.